Imaging Agents

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One-Pot Synthesis of Highly Magnetically Sensitive Nanochains Coated with a Highly Cross-Linked and Biocompatible Polymer**

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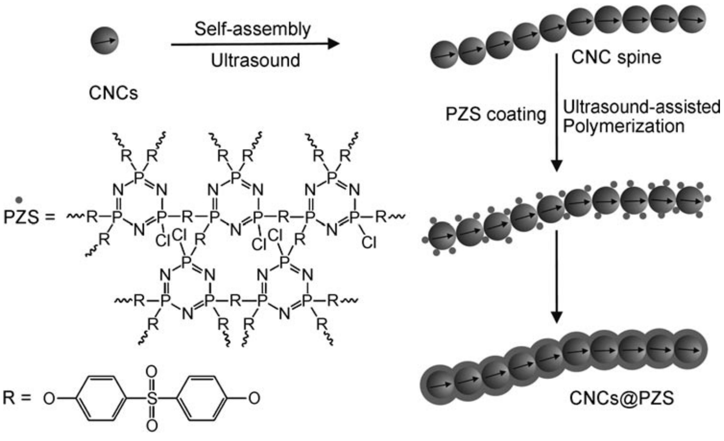
The organization and functionalization of nanoparticles into hierarchically ordered nanomaterials has attracted great interest because of their unique structures and their electronic,[1] optical,[2] and magnetic properties.[3] Among the synthetic techniques used, organization of magnetic nanoparticles into one-dimensional (1D) nanostructures is particularly intriguing for both fundamental research and practical applications.[3] Two basic strategies have been proposed to obtain 1D magnetic nanostructures, including the use of nanostructured templates[4] or through a controlled self-assembly process.[5] For the latter approach, magnetic dipole-directed assembly represents a versatile method to fabricate magnetic chainlike structures.[5a] However, owing to the weak or negligible anisotropic dipolar interaction between the magnetic building blocks, these ordered structures can hardly be maintained after removal of the external field.[5e] In this regard, although well-defined block copolymers[3] or end-functional polymers[5b,d] have been utilized to stabilize 1D magnetic chains, they suffer from disintegration during rinsing with a good solvent.[6] Cross-linked polymeric shells are able to tackle this obstacle,[6] but the synthesis of these organic cross-linkable surfactants are complicated and time-consuming.[7] The development of a facile method to directly obtain 1D magnetic nanochains coated with cross-linked polymers is however still attractive.

Herein we present a facile one-pot synthesis of 1D magnetic nanochains coated with highly cross-linked polymer. Fe_3O_4-based colloidal nanocrystal clusters (CNCs) were selected as building blocks for 1D nanochains owing to the combination of paramagnetism and remarkable magnetic response.[8] The highly cross-linked polymer poly(cyclotriphosphazene-*co*-4,4′-sulfonyldiphenol) (PZS) was chosen as the shell for the stabilization and functionalization of the 1D nanochains, and it endows the nanochains good water dispersibility, biocompatibility, and tailored surface chemistry.[9] On account of the 1D assembly and the PZS coating, the nanochains display an enhanced magnetic resonance (MR) sensitivity and biocompatiblility.

The synthesis of CNCs@PZS nanochains is illustrated in Scheme 1. The Fe_3O_4 CNCs were first prepared according to a modified procedure.[8a] The CNCs were then assembled into 1D nanochains with the assistance of ultrasonic irradiation, whilst PZS were generated and coated the CNC nanochains by the polycondensation of hexachlorocyclotriphosphazene (HCCP) and 4,4′-sulfonyldiphenol (BPS; Supporting Information, Scheme S1). The resulting solids were easily collected with a magnet, then washed with tetrahydrofuran (THF) and deionized water, and finally dried under vacuum to give a black powder for storage under air (see the Experimental Section for details). Because the CNC nanochains were firmly "sandwiched" inside the highly cross-linked structure of PZS shell, the 1D magnetic chains appeared to be structurally robust and well preserved after multiple rinsing with organic solvent. Furthermore, the CNCs@PZS nanochains could be well re-dispersed in water and other polar organic solvents, such as THF, acetone, and ethanol, thus allowing potential further surface modification in both aqueous and organic media.



Scheme 1. Preparation of one-dimensional chainlike colloidal nanocrystal clusters with a poly(cyclotriphosphazene-*co*-4,4′-sulfonyldiphenol) shell (CNCs@PZS).

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Transmission electron microscopy (TEM) was employed to investigate the morphology and structure of the as-synthesized materials. Figure 1 a,b demonstrates that the CNCs are very monodisperse, with an average diameter of (192.0 ± 20.0) nm, which is in accordance with that in scanning electron microscopy (SEM) images ((194.9 ± 20.0) nm; Supporting Information, Figure S1a, b). A magnified TEM image (Supporting Information, Figure S1d) shows that each CNC is composed of hundreds of interconnected Fe_3O_4 nanocrystals with a size of around 20.0 nm. Figure 1 c,d show typical SEM images of as-synthesized CNCs@PZS at different magnifications. These images indicate that most of the CNCs@PZS have a chainlike morphology instead of isolated nanoballs, and the good junction of adjacent CNCs can be clearly seen in Figure 1 d. The core@shell structure of as-synthesized CNCs@PZS nanochains were further investigated by TEM. As illustrated in Figure 1 e,f, the black CNCs formed 1D nanochains by head-to-tail interactions in a continuous thin PZS shell (gray color). The thickness of the PZS shell is estimated to be about 20 nm in Figure 1 f. As a control experiment, we also carried out the polymerization of HCCP and BPS in the absence of CNCs. In this case, only PZS microspheres were obtained and no nanochains could be observed (Supporting Information, Figure S2). This result indicates that the pre-organization of linear CNC chains before the undergoing coating with PZS was crucial to the formation of nanochains. The pre-formed CNC spines acts template and the PZS shell encircled the spines, leading to formation of nanochains (Figure 1 c–f).

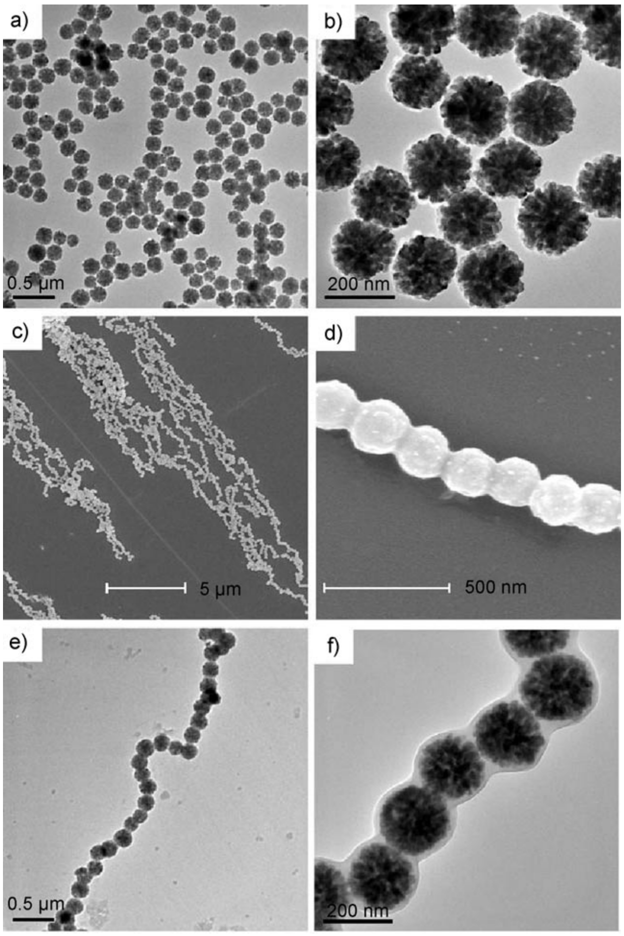

Figure 1. a, b) TEM images of CNCs that are about 192.0 nm in diameter; c, d) SEM and e, f) TEM images of CNCs@PZS chains with circa 20 nm shell thickness.

FTIR spectroscopy further confirmed the successful formation of CNCs@PZS. The new absorption at 941 cm^{-1} can be assigned to the P-O-Ar band, suggesting the occurrence of polycondensation between HCCP and BPS (Supporting Information, Figure S3a). The peaks at about 3500 cm^{-1} may be mainly attributed to the stretching vibration of the phenolic hydroxy groups, which offer high surface activity to bind other biomolecules or drugs (Supporting Information, Figure S3b). We also measured the zeta potential of CNCs and CNCs@PZS to provide further evidence of phenolic hydroxy groups on the surface of CNCs@PZS nanaochains. The zeta potential of CNCs@PZS significantly decreased to (−36.0 ± 4.6) mV (from (5.0 ± 4.5) mV of CNCs) because of the ionization of phenolic hydroxy groups, implying the increase of negative charge density on the surface (Supporting Information, Table S1). Obviously, the electrostatic repulsion among CNCs@PZS nanochains is of benefit to the colloidal stability in water and other polar solvents to form homogeneous dispersions.

Energy-dispersive X-ray spectrometry (EDS) was also used to ascertain that the CNCs@PZS nanochains consist of the elements C, S, P, Cl, O, and Fe (Supporting Information, Figure S3c). A selected-area electron diffraction (SAED) pattern and X-ray diffraction (XRD) reveal a face-centered cubic phase of CNCs (Supporting Information, Figures S1c, S3d). Along with the characteristic reflection peaks of CNCs, the XRD pattern of CNCS@PZS illustrates a new, very broad diffraction peak at about 23°, indicating the amorphous nature of the PZS layer (Supporting Information, Figure S3d).

One of the most important requirements of magnetic materials is their highly magnetic sensitivity and good magnetic manipulation when used in biotechnology and biomedicinal applications, such as magnetothermal therapy and DNA separation. Figure 2 a shows that the magnetization saturation values (M_s) of bare CNCs and CNCs@PZS are 70.2 and 62.4 emu g^{-1}, respectively. The very high M_s values of CNCs suggests good crystalline nature of magnetite Fe_3O_4 as the building blocks of CNCs (also confirmed by XRD and SAED). The M_s values of the CNCs@PZS is somewhat lower than that of CNCs owing to the lower magnetic component in the composite. Remarkably, the magnified low-field hysteresis curve reveals the quasi-superparamagnetic property of CNCs@PZS that exhibits very little remanence effects at 300 K (Figure 2 b). The high M_s values and quasi superparamagnetic property of CNCs@PZS nanochains may offer fast and easy magnetic manipulation, as was confirmed by a manipulation experiment (Supporting Information, Video S1). We observed that CNCs@PZS could be swiftly isolated from their dispersion towards the applied magnetic field, whilst the solids could be re-dispersed by mechanical shaking once the magnetic field was removed. Of particular

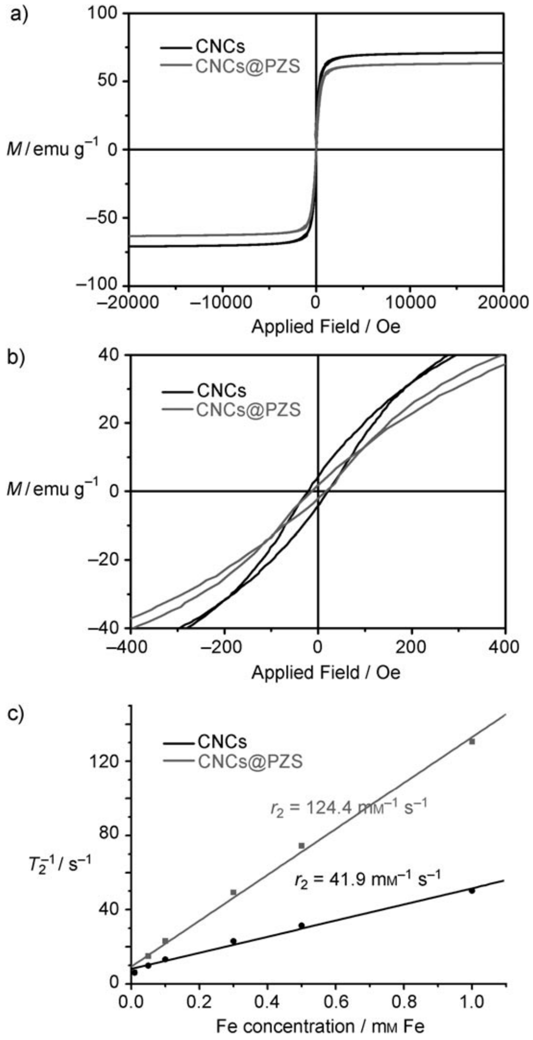

Figure 2. a, b) Magnetization curves and magnified curves of CNCs and CNCs@PZS measured at 300 K, c) T_2 relaxation rates (as $1/T_2$) as a function of iron concentration (mM) for CNCs and CNCs@PZS in aqueous solutions (1.5 T, 298 K).

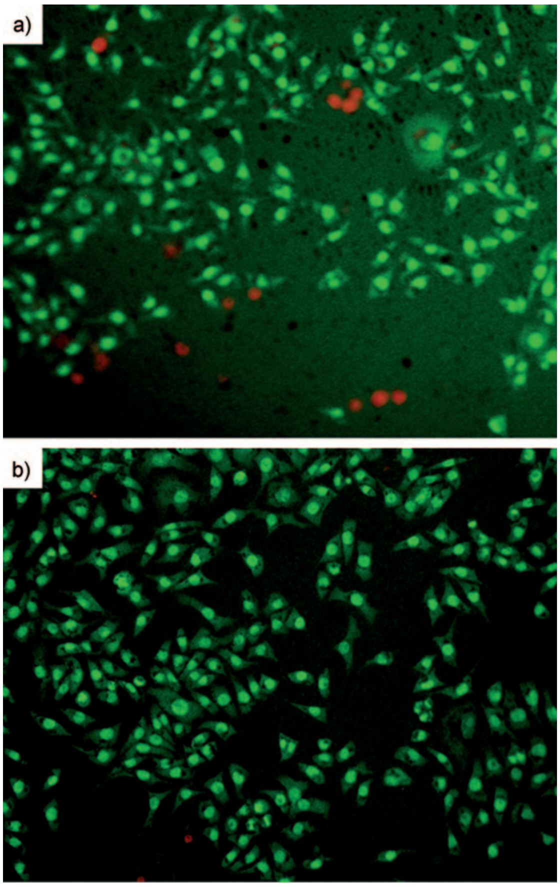

Figure 3. Fluorescence microscope images of T3 cells cultured with a) CNCs and b) CNCs@PZS for 24 h. The cells were stained with acridine orange/ethidium bromide.

interest is that CNCs@PZS nanochains have a much higher MR contrast ($r_2 = 124.4\,\text{mM}^{-1}\text{s}^{-1}$) than that of CNCs ($r_2 = 41.9\,\text{mM}^{-1}\text{s}^{-1}$; Figure 2c). The increased MR contrast of CNCs@PZS nanochains is probably attributed to enhanced spin–spin relaxation of water molecules caused by the 1D assembly and the PZS coating. Thus, it seems that CNCs@PZS nanochains are promising candidates as higher efficiency T_2 contrast agents for a variety of MR imaging applications.

Along with the high magnetic response of CNCs@PZS, good biocompatibility is also key to biotechnology applications. The bare CNCs and 1D CNCs@PZS nanochains were each cultured with mouse fibroblast (T3) cells, and the cell viability was studied by acridine orange/ethidium bromide (AO/EB) double staining.[10] Generally, healthy cells have green nuclei and uniform chromatin with an intact cell membrane, whereas the cells in necrosis or at a late stage of apoptosis have red nuclei with a damaged cell membrane. As demonstrated in Figure 3a, some cells cultured with CNCs were in necrosis with red nuclei after culturing for 24 h. In contrast, fewer necrotic cells were observed on a CNCs@PZS sample (Figure 3b), suggesting that PZS shells indeed improved the biocompatibility.

To demonstrate the generality of the synthetic method, we also prepared another two CNCs, positively charged CNCs with about 152 nm diameter and negatively charged CNCs with about 262 nm diameter (see the Supporting Information for the synthesis). They could also be assembled and coated with PZS shells to form CNCs@PZS nanochains (see the Supporting Information, Figures S4, S5). Interestingly, by tuning the mass ratio of the CNCs to the precursors HCCP and BPS of PZS, the thickness of the PZS shells could be controlled. For instance, when the mass of HCCP and PBS monomers was increased to three- or five-fold, the thickness of the resulting PZS can be tuned from about 30 nm to about 180 nm (Supporting Information, Figures S4d–4f, S5d–5f). Almost all of the nanochains have a closed end, and neither bare CNCs nor isolated CNCs@PZS spheres are present in these TEM images, thus demonstrating a highly efficient approach for 1D nanostructure synthesis. The formation and topology of the nanochains are obviously influenced by the intensity of ultrasonic irradiation. Long CNCs@PZS nanochains could be formed at 30 W irradiation (Figure 1; Supporting Information, Figure S4), whereas short nanochains were obtained at 50 W irradiation (Supporting Information, Figure S5). If the PZS was synthesized in the CNC dispersions by mechanical stirring in the absence of ultrasonic assistance, only very short assemblies and nanoballs could be observed (Supporting Information, Figure S6), suggesting that the ultrasonic irradiation may play an important role in the assembly of CNCs into nanochains.

On the basis of the above results, the formation of CNCs@PZS can be attributed to a mechanism involving the dipole-directed self-assembly of CNCs as the hard template, followed by PZS coating. First, the CNCs could self-organize by head-to-tail orientation into 1D chainlike CNC spines from magnetic dipolar interactions under ultrasonic assistance. Second, under the ultrasonic irradiation, polycondensation of HCCP and PBS was induced by addition of triethylamine (TEA) as base into the mixture suspension. At the initial stage of the reaction, large numbers of nanoscale PZS colloids were produced, which then diffused to and coated the surface of the pre-assembled CNC spines because of the strong affinity between PZS and CNCs. With the progress of the polymerization reaction, the PZS nanoshells continually encircled the CNC cores. Meanwhile, the cross-linking between PZS colloids led to the formation of 1D anisotropic nanochains with CNC spine as the core and PZS as the shell. Because of the layer-by-layer coating process, the thickness of PZS shell could be easily controlled (Supporting Information, Figures S4, S5). The coating process of PZS may be similar with that of nanotubes[9a] and cables.[9b]

In conclusion, we have developed a facile approach to synthesize highly magnetically sensitive 1D nanochains wrapped with a highly cross-linked polymer. The nanochains possess very high, quasi-superparamagnetic magnetization saturation values and enhanced MR contrast whilst inheriting all the advantages from PZS, that is, long-term colloid stability, favorable water dispersibility, good biocompatibility, and tailored surface chemistry for binding biomolecules or drugs, thereby leading to great potential in magnetic and biomedical applications.

Experimental Section

Fe_3O_4 CNCs were prepared according to a modified procedure.[8a] $FeCl_3 \cdot 6\,H_2O$ (1.35 g, 5 mmol) was dissolved in ethylene glycol (40 mL) to form a clear solution, followed by the addition of sodium acetate (3.6 g) and 1,2-ethylenediamine (10 mL). The mixture was stirred for 30 min and then sealed in a Teflon-lined stainless-steel autoclave (50 mL capacity). The autoclave was heated at 200 °C for 8 h. After cooling to room temperature, the black products were collected by filtration, washed with ethanol (3 × 15 mL), and dried at 45 °C under vacuum overnight.

Synthesis of CNCs@PZS nanochains: In a typical experiment, CNCs (100 mg), HCCP (4 mg, 11.5 mmol) and BPS (9 mg, 36.0 mmol) were added into a 50 mL round-bottom flask. A mixture of THF and anhydrous alcohol (30 mL, 1:1 by volume) was subsequently added. After ultrasonic irradiation for 10 min (30 W, 40 kHz), 0.5 mL of triethylamine was injected into the mixture. The solution was then maintained at room temperature for 6 h under ultrasonic irradiation (30 W, 40 kHz). As soon as the reaction was complete, the resulting solids were collected by a magnet, washed with deionized water (3 × 15 mL) and THF (3 × 15 mL), and dried at 45 °C under vacuum overnight.

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